Crystallographic and Biochemical Analysis of Recombinant and Mutant Cytochrome *ba_3* Oxidase from *Thermus thermophilus*

James A. Fee, Laura M. Hunsicker-Wang, Ying Chen, and C. David Stout

Department of Molecular Biology, The Scripps Research Institute

Cytochrome *c* oxidase is an integral-membrane protein that performs the reduction of dioxygen to H_2O concomitantly with proton translocation. Development of a recombinant expression and purification system for cytochrome *ba_3* oxidase, isolated from the thermophilic eubacterium, *Thermus thermophilus*, has yielded native-like recombinant and mutant forms of the enzyme. Analysis of structure-based sequence alignment of four such oxidases revealed a dramatically small number of residues that can truly be considered as essential for cytochrome *c* oxidase and proton pumping functions. Our recombinant protein crystallized under similar conditions and in the same space group ($P4_32_12$) as the native protein. A novel cryoprotection scheme was developed using a combination of traditional cryoprotectants and incubation under oil to produce high resolution data for the recombinant protein. The recombinant structure was solved and refined to 2.3 Å, with R of 21.7% and R_{free} of 23.7%. The cell constants for these crystals are larger than for the native protein, apparently deriving from increased ordering of the N-terminus and an internal loop (residues 495-500) in subunit I. The presence of a glycerol molecule near the active site, and an apparently shorter Fe_{a3} – N His 384 distance are two notable differences between the native and recombinant structures. Crystals of the I-Y237F and I-Y237H mutant proteins have also been achieved. *Supported by NIH grant GM35342*.